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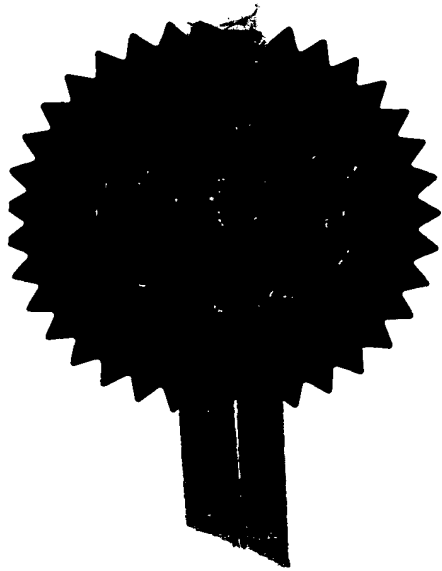
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1. Your reference

REP05969GB

2. Patent application number

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9922596.3

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Microscience Limited
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

GB

758368500-06

4. Title of the invention

PROTEIN AND COMPOSITIONS
CONTAINING IT

5. Name of your agent (if you have one)

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Broadgate House
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11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

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Date

23 September 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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PROTEIN AND COMPOSITIONS CONTAINING IT

Field of the Invention

This invention relates to one protein, to vaccines
5 containing it, and to its use in therapy, for immunisation.

Background to the Invention

Group B Streptococcus (GBS), also known as
Streptococcus agalactiae, is the causative agent of various
conditions. In particular, GBS causes:

10 *Early onset neonatal infection.*

This infection usually begins *in utero* and causes
severe septicaemia and pneumonia in infants, which is
lethal if untreated and even with treatment is associated
with a 10-20% mortality rate.

15 *Late onset neonatal infection.*

This infection occurs in the period shortly after
birth until about 3 months of age. It causes a
septicaemia, which is complicated by meningitis in 90% of
cases. Other focal infections also occur including
20 osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

Adult infections.

These appear to be increasingly common and occur most
commonly in women who have just delivered a baby, the
25 elderly and the immunocompromised. They are characterised
by septicaemia and focal infections including
osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

Urinary tract infections.

30 GBS is a cause of urinary tract infections and in
pregnancy accounts for about 10% of all infections.

Veterinary infections.

GBS causes chronic mastitis in cows. This, in turn,
leads to reduced milk production and is therefore of
35 considerable economic importance.

GBS infections can be treated with antibiotics.
However, immunisation is preferable. It is therefore

desirable to develop an immunogen that could be used in a therapeutically-effective vaccine.

Summary of the Invention

According to the present invention, a GBS gene
5 sequence for the protein Ornithine Carbamoyltransferase (OCT) has been found which represents an outer surface protein.

In one aspect of the invention, the use of this protein in a recombinant protein vaccine is described.
10 This vaccine may be administered to females either prior to, or during pregnancy to protect mother and neonate against infection by GBS.

The gene sequence may be first genetically altered to increase the antigenicity of the encoded protein.

15 Brief Description of the Drawings

The invention will now be described in detail with reference to the accompanying figures, wherein:

Figure 1 shows the nucleotide sequence and the deduced amino acid sequence of the GBS OCT;

20 Figure 2a shows the generated peptide sequences for an isolated peptide digest of the protein of the invention; and

Figure 2b shows the oligonucleotide sequences derived from the peptide sequences.

25 Description of the Invention

Because of its extracellular or cell surface location, the protein of the present invention may be a suitable candidate for the production of therapeutically-effective vaccines against GBS. The term "therapeutically-effective"
30 is intended to include the prophylactic effect of the vaccines. For example, a recombinant protein may be used, as an antigen for direct administration to a patient. The protein may be isolated directly from GBS or expressed in any suitable expression system, e.g. *Lactococcus lactis*.
35 It is preferably administered with an adjuvant, e.g. alum.

The protein may be a mutant protein in comparison to wild-type protein, a fragment of the protein or a

combination of different fragments, provided an effective immune response is generated.

An alternative approach is to use a live attenuated GBS vaccine. This may be produced by deleting the gene
5 that encodes the protein. Preferably, the GBS strain comprises additional virulence gene mutations.

The protein (or fragments thereof) of the present invention may also be used to produce monoclonal and polyclonal antibodies for use in passive immunisation.

10 In a further embodiment of the invention, the protein or corresponding polynucleotide may be used as a target for screening potentially useful drugs, especially antimicrobials. Suitable drugs may be selected for their ability to bind to the protein to exert their effects.
15 Assays for screening for suitable drugs and which make use of the protein of the invention will be apparent to those skilled in the art.

Although the protein has been described for use in the treatment of patients, veterinary uses of the protein are
20 also considered to be within the scope of the present invention. In particular, the protein or the vaccines may be used in the treatment of chronic mastitis, especially in cows.

The present invention is described with reference to
25 Group B Streptococcal strain M732. However, all the GBS strains and many other bacterial strains are likely to include related proteins having amino acid sequence homology with the protein of M732. Organisms likely to contain the proteins include, but are not limited to, *S.*
30 *pneumoniae*, *S. pyogenes*, *S. suis*, *S. milleri*, Group C and Group G *Streptococci* and *Enterococci*. Vaccines to each of these may be developed in the same way as described for
GBS.

Preferably, the proteins that may be useful for the
35 production of vaccines have greater than 40% sequence similarity with the protein of M732. More preferably, the proteins have greater than 60% sequence similarity. Most

preferably, the proteins have greater than 80% sequence similarity.

The protein of the present invention was identified as follows:

5 Todd-Hewitt Broth was inoculated with GBS and allowed to grow overnight at 37°C. The cells were harvested by centrifugation and washed with Phosphate Buffered Saline (PBS). The cells were resuspended in an osmotic buffer (20%(w/v) Sucrose, 20mM Tris-HCl pH 7.0, 10mM MgCl₂)
10 containing protease inhibitors (1 mM PMSF, 10 µM Iodoacetic Acid, 10 mM 1,10-Phenanthroline, 1 µM Pepstatin A) and Mutanolysin at a final concentration of 4 Units per microlitre. This was incubated (shaking) at 37°C for 2 hours.

15 Cells and debris were removed first by high speed centrifugation, then ultra-centrifugation for 1 hour. The resultant supernatant containing cell wall proteins was concentrated under pressure using an ultrafiltration device (10,000 molecular weight cut-off).

20 The sample was dialysed against ultra high quality water and lyophilised. After resuspension in loading buffer, the proteins were separated by preparative 2-Dimensional-Gel Electrophoresis. Following electrophoresis an individual spot was chosen for study. The spot was
25 subjected to in-gel tryptic digestion. The resulting peptides were extracted from the gel and purified using microbore RP-HPLC. Fractions were collected every 45 seconds and a portion of these consistent with the regions of UV absorbance were analysed by Delayed Extraction-Matrix
30 Assisted Laser Desorption-Time of Flight Mass Spectrometry (DE-MALDI-TOF-MS). Peptides not observed in a blank preparation were then subjected to sequencing using
Nanospray-MS/MS

The Peptide Sequences obtained are shown in Figure 2a.

35 Using this information, degenerate oligonucleotides were designed to be used in a polymerase chain reaction (PCR) to amplify the DNA segment lying between the peptide

sequences identified. The sequences of these oligonucleotides is shown in Figure 2b.

PCR amplification resulted in the production of an (approximately) 250 base pair fragment, which was cloned
5 into the PCR 2.1-TOPO vector (Invitrogen BV, Netherlands) according to manufacturers protocol. This plasmid was termed MS4. The cloned DNA fragment was sequenced (Figure 1) and the deduced amino acid sequence was used to search protein databases. Results of this search are shown in
10 Table 1.

As shown in Table 1, homologues to the GBS MS4 gene product can be identified in *Clostridium perfringens*, *Haemophilus influenzae*, *Neisseria flavescens* and *Thermatoga maritima*. In all cases the homologues are the genes for
15 Ornithine Carbamoyltransferase (OCT). In eukaryotic systems this enzyme catalyses the second step in the Urea cycle, the conversion of ornithine to citrulline, a reaction requiring carbomyl phosphate. In prokaryotes, ODC is one of the three enzymes involved in Arginine Deaminase
20 activity - a system which protects bacteria from acid damage. In particular, ODC is responsible for the conversion of citrulline to ornithine and carbamoyl phosphate (the opposite role to that in eukaryotes) (Casiano-Colon, A and Marquis, R. E. 1988. Appl. Environ.
25 Microbiol. 54: 1318-1324, Cunin, R. et al. 1986. Microbiol. Rev. 50: 314-352).

Table 1. Database search results for MS4

Organism	Protein Accession	DNA Accession	Gene Name	% Simila rity	% Identity	Alignment Length
<i>Clostridium perfringens</i>	Q46169	X97768	Ornithine Carbamoyltransferase	80	58	246
<i>Haemophilus influenzae</i>	P44770	1573585	Ornithine Carbamoyltransferase	77	57	246
<i>Neisseria. flavescens</i>	Q01323	X64872	Ornithine Carbamoyltransferase	79	55	246
<i>Thermatoga maritima</i>	P96108	Y10661	Ornithine Carbamoyltransferase	63	48	246

CLAIMS

1. A protein comprising an amino acid sequence encoded by the polynucleotide defined as MS4 in Figure 1 or a homologue thereof with at least 60% sequence homology.
 - 5 2. A protein according to claim 1, obtainable from the Group B streptococcal strain M732.
 3. A protein according to claim 1 or claim 2, wherein MS4 comprises the nucleotides 1-252.
 4. A protein according to any of claims 1 to 3, for use
10 in a method of therapy.
 5. A polynucleotide which encodes a protein according to any preceding claim, its complement, or a fragment thereof.
 6. The use of a bacterial protein according to any of claims 1 to 4, in the manufacture of a vaccine to treat
15 bacterial infection.
 7. The use according to claim 6, wherein the infection is a Group B streptococcal infection.
 8. The use according to claim 6 or claim 7, wherein the infection is a focal infection.
 - 20 9. The use according to claim 6 or claim 7, wherein the infection is a urinary tract infection.
 10. Use of a product according to any of claims 1 to 5, for screening potential antimicrobial drugs.
 11. An antimicrobial drug selected using the products as
25 defined in claim 10.
 12. A vaccine comprising a product according to any of claims 1 to 5.
 13. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene codes for a
30 protein according to any of claims 1 to 4.
 14. An antibody raised against a protein according to any of claims 1 to 4.
-

Figure 1

Complete sequence of Group B Streptococcus Ornithine
Carbamoyltransferase

```

      10              30              50
ATGACACAAGTATTTCAAGGACGTAGTTTCTTAGCAGAAAAAGATTTTTC
M T Q V F Q G R S F L A E K D F S

      70              90
TCGTGAGGAATTTGAATATCTTATTGATTTTTTCAGCTCATTTAAAAGACC
R E E F E Y L I D F S A H L K D L

     110             130             150
TTAAAAAACGTGGTGTTCCTCATCATTATCTTGAAGGTAAAAATATTGCT
K K R G V P H H Y L E G K N I A

     170             190
CTCTTATTTGAAAAACATCTACTCGTACTCGCGCAGCCTTTACAACGTC
L L F E K T S T R T R A A F T T A

     210             230             250
AGCAATTGACCTAGGCGCTCATCCGGAATACCTTGGTGCAAATGATATTC
A I D L G A H P E Y L G A N D I Q

     270             290
AACTTGGTAAAAAAGAATCAACAGAAGATACTGCTAAGGTTTTAGGACGT
L G K K E S T E D T A K V L G R

     310             330             350
ATGTTTGATGGTATTGAATTCCGTGGTTTTAGCCAAAGAATGGTTGAAGA
M F D G I E F R G F S Q R M V E E

     370             390
GCTTGCTGAATTTTCTGGAGTACCTGTCTGGAATGGTTTAACAGATGAAT
L A E F S G V P V W N G L T D E W

     410             430             450
GGCATCCAACACAAATGCTAGCTGACTACCTTACTATCAAAGAAAAC TTC
H P T Q M L A D Y L T I K E N F

     470             490
GGTAACTTGAAGGTATTACTCTTGTTTACTGTGGTGACGGACGTAACAA
G K L E G I T L V Y C G D G R N N

     510             530             550
TGTTGCCAACTCGCTTTTAGTGGCTGGGACTTTGATGGGGGTCAATGTAC
V A N S L L V A G T L M G V N V H

-----
     570             590
ACATCTTTTCTCCAAAAGAACTTTTYCCWGCTGAAGAGATTGTTAAATTG
I F S P K E L F P A E E I V K L

     610             630             650
GCTGAAGGATATGCCAAaGAATCTGGGGCTCACGTTCTCGTTACTGATAA
A E G Y A K E S G A H V L V T D N

```



TGTAGACGAAGCTGTAAAGGGAGCAGACGTCTTTTACACTGATGTCTGGG
V D E A V K G A D V F Y T D V W V

710 730 750
TATCGATGGGAGAAGAAGATAAGTTCAAAGAACGCGTTGAACTTCTTCAA
S M G E E D K F K E R V E L L Q

770 790
CCATATCAAGTAAACATGGAAGTATTAAAAAGCTAATAATGATAATCT
P Y Q V N M E L I K K A N N D N L

810 830 850
TATCTTCTTACACTGCTTACCTGCATTCCATGATACAAATACCGTTTATG
I F L H C L P A F H D T N T V Y G

870 890
GCAAAGACGTCGCTGAAAAATTTGGGGTCAAGGAAATGGAAGTTACTGAT
K D V A E K F G V K E M E V T D

910 930 950
GAAGTCTTCCGTAGCAAATATGCTCGTCATTTGACCAAGCTGAAAATCG
E V F R S K Y A R H F D Q A E N R

970 990
TATGCACACTATTAAAGCTGTAATGGCTGCAACCCTTGGAATCTTTTCA
M H T I K A V M A A T L G N L F I

1010
TTCCAAAAGTTTAA
P K V *

Figure 1 (cont.)



Figure 2a. Generated Peptide Sequences for MS4

PAFHD

FPAEE(L/I)VK

Figure 2b. Oligonucleotide sequences designed from
Peptide sequences in Figure 2a

TTTCCAGCTGAAGA

RTCRTGRAADGCHGG

PT 9904376
LW Jennings & Every
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